

In - Vitro Evaluation of Antihelminthic Activity of Extracts of Ocimum Gratissimum and Coriandrum Sativum

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Abstract: *The present study investigated anthelmintic resistance and the high cost of conventional anthelmintic drugs it leads to the evaluation of medicinal plants as an alternative source of anthelmintics. The anthelmintic activity of ethanolic leaf extracts of Coriandrum sativum and ocimum gratinissimum. In - vitro experiments were conducted to determine the possible anthelmintic effects. Data in the table 1 reveals that the extracts of ocimum gratinissimum plant took the less time to cause paralysis of the earthworm than that of coriandrum sativum extract. The three concentrations of Coriandrum sativum and ocimum gratinissimum (50, 100, and 150 mg/ml) showed paralysis at 87.00, 42.33 and 32.33 min and death at 191.66, 146.00 and 88.33 min and (50, 100, and 150 mg/ml) paralysis at 97.33, 72.00 and 48.66 min and death at 215.00, 152.00 and 109.66 min of the worms. The standard drug, Piperazine citrate showed paralysis at 21.66 min and death after 72.33 min at 15mg/ml concentration and control was used as water. From the above result, it is clear that extracts of ocimum gratinissimum and Coriandrum sativum plants have significant anthelmintic activity in dose dependent manner when compared with standard anthelmintic drug. It can be concluded that the active constituents responsible for anthelmintic activity present in the both extracts of Coriandrum sativum and ocimum gratinissimum plants.*

Keywords: Anthelmintic activity, Coriandrum sativum and ocimum gratinissimu plants, Pheritima posthuma, Ascaridia galli, Piperazine citrate.

1. Introduction

Coriandrum sativum and ocimum gratinissimum plants have been used as an excellent source of medicine from the outset, which established a foundation of traditional medicine. Such traditional medicinal plants play a vital role in addressing the global health needs of today and their use will increase in the future. Belongs to the family Coriander (*Coriandrum sativum* L.) being an annual herb is most commonly used for seasoning purpose also known as *Coriandrum sativum* L. and *ocimum gratinissimum* family Lamiaceae is known for its anthelmintic activity. In this study, a number of *Coriandrum sativum* and *ocimum gratinissimum* extracts were tested for antihelminthic activity. I was picked *Coriandrum sativum* and *ocimum gratinissimum* having antihelminthic activity, based on the plants with different concentrations solvents were collected to extract samples. *Coriandrum sativum* and *ocimum gratinissimum* plants contain many chemical components that are responsible for the achievement of various physiological and therapeutic responses. The ethanolic extracts were then subjected to the various qualitative tests for the detection of *Coriandrum sativum* and *ocimum gratinissimum* plants constituents like alkaloids, glycosides, tannins, carbohydrates, coumarins, saponins, flavanoids, proteins etc. Owing to the side effect of chemical drugs, the use of medicinal plant extracts for the treatment of human diseases has greatly increased in the past few decades. The phytochemical in plants act as a medicine; therefore, plants have been used as a source of medicine for thousands of years. The three concentrations of *Coriandrum sativum* and *ocimum gratinissimum* (50, 100, and 150 mg/ml) showed

paralysis at 87.00, 42.33 and 32.33 min and death at 191.66, 146.00 and 88.33 min and (50, 100, and 150 mg/ml) paralysis at 97.33, 72.00 and 48.66 min and death at 215.00, 152.00 and 109.66 min of the worms. The standard drug, Piperazine citrate showed paralysis at 21.66 min and death after 72.33 min at 15mg/ml concentration and control was used as water. From the above result, it is clear that extracts of *ocimum gratinissimum* and *Coriandrum sativum* plants have significant anthelmintic activity in dose dependent manner when compared with standard anthelmintic drug. It can be concluded that the active constituents responsible for anthelmintic activity present in the both extracts of *Coriandrum sativum* and *ocimum gratinissimu* plants.

In vitro comes from the Latin term "in glass" and refers to studies of biological properties that are done in a test tube (i. e. under glass) rather than in a human or animal. Studies that are in - vitro (Latin: in glass; often not italicized in English) are performed with the cells or biological molecules studied outside their normal biological context; for example, proteins are examined in solution, or cells in artificial culture medium. Examples of in vitro studies include: isolation, growth and identification of microorganisms; cells derived from multi - cellular organisms (cell culture to tissues culture); sub cellular components (e. g. mitochondria or ribosome's); and the commercial production of antibiotics and other pharmaceutical products.

Helminthic is a worldwide and one of the common diseases of all ages especially in third world countries. Parasitic infection including Helminthiasis is a critical serious problem in the tropical regions including the Asian and African countries which affects more than 2.5 billions of

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people worldwide. Helminthes produce serious problem in human beings and other animals around the world specifically to the third world countries¹. Different type of helminthes infects the human and animals out of which intestinal round worms *Pheretima posthuma* (Annelid) are most common. Approximately 200 million people suffer severe morbidity associated with these parasites and half of which are school - going children affected by massive infections. Variety of several clinical symptoms arises due to this infection include dysentery, diarrhea, nausea, vomiting, loss of appetite, loss of weight, acidity and anemia². Other sign and symptoms of helminthic infections include respiratory symptoms, dermatological consequences and epilepsy as a result of neurocysticercosis. Helminthic infections may also subvert immune responses to pathogens of other diseases such as tuberculosis, HIV, and malaria². Although the majority of infections are due to the worms generally limited to tropical regions, they can also occur to travelers who have visited those areas and some of them can develop in temperate climates³. Helminthiasis is a disease in which a part of the body is infested with worms like as pinworm, roundworm or tapeworm. Typically, the worms present in the gastrointestinal tract but may also reside into the liver and other organs, infected peoples are excrete helminth eggs in their faeces, which then contaminate the soil in areas with inadequate sanitation⁴. Other peoples can be infected by ingesting eggs or larvae in contaminated food, or through penetration of the skin by infective larvae in the soil (hookworms). Parasitic diseases can cause severe morbidity, including filariasis (a cause of elephantiasis), onchocerciasis (river blindness), and schistosomiasis⁵. As per the WHO survey only synthetic drugs are sometimes used in the treatment of helminth infestations in human beings but these synthetic drugs are out of reach of millions of people and have a lot of side effect. In view of this, an attempt has been made to study the anthelmintic activity of herbal drug.

Development of resistance to most of the commercially available anthelmintics drugs are became a severe problem worldwide. Sometimes, these drugs are unaffordable, inaccessible or inadequately available to the resource poor farmers of the developed and developing countries⁶. These factors paved the way for herbal remedies as alternative anthelmintics⁷. Therefore the evaluation of the activities of medicinal plants claimed for possessing the anthelmintic property is getting attention these days⁸. Screening and proper evaluation of the claimed medicinal plants as anthelmintics could offer possible alternatives that may be both sustainable and environmentally acceptable⁹. *Coriandrum sativum* and *ocimum gratissimum* is fast - growing small tree belonging to family apiaceous and lamiaceae. The plant is native to India, Pakistan, Laos, Myanmar, Vietnam, China, and Bhutan. The plants *Coriandrum sativum* and *ocimum gratissimum* is located at hilly forests, in ravine and on hill slopes. The plants are known as (coriandrine and Vriddhutulsi in Sanskrit)¹⁰. The leaves were used as digestive food purpose. Leaves are applied to ulcers, skin diseases and in headache. Fruit is used as urinary passage, lung and spleen diseases, astringent, anthelmintic, diuretic, and demulcent, expectorant. Powdered kernel mixed with oil is a remedy in ringworm. Seeds are anthelmintic. Barks are used in throat infection.

Root for venerably diseases. The plant contains chemical constituents like's fatty acids, phenolic acids, flavonoids, cyanogenetic glycosides, and benzoquinones^{11, 12}.

Coriander

Coriander (*Coriandrum sativum* L.) being an annual herb is most commonly used for seasoning purpose. Its plant seeds, leaves and roots are edible, although they have very distinct flavors and uses. The herb has a light and fresh flavor. Coriander can be used as whole plant and can be processed because of its perishable nature of leaves and to increase the palatability of ripe fruits (seeds) before using it as flavoring agent in different food preparations. Whole plant of coriander mainly fresh leaves and ripe fruits are used for culinary purposes. Coriander leaves have different taste than its seeds, with citrus over tones Coriander is very low in saturated fat however, contains good amount of linoleic acid which is a good source of α - tocopherol and vitamin K. Leaves of plant are rich source of vitamins while seeds are rich in polyphenols and essential oils. Coriander taste is devoted to its essential oil comprising a significant content of linoleic and furanocoumarins (coriandrine and dihydrocoriandrine). Coriander is also well known for its antioxidant, anti - diabetic, anti - mutagenic, anti - anxiety and antimicrobial activity along with analgesic and hormone balancing effect that promotes its use in foods due to numerous health benefits and its protective effect to preserve the food for longer period.



Coriander (*Coriandrum sativum* L.)

It is woody at the base and has an average and narrowly ovate, usually 5 - 13 cm long and 3 - 9 cm wide. It is scented shrub with lime green leaves. The plant is consumed by the logos as a leafy and vegetable and nutritional importance of this plant center on its usefulness as a seasoning because of its aromatic flavor. It is also used by globes in this management of the baby's cord. It is believed to keep the baby's cord wounds surface sterile. It is used in the treatment of fungi infection, fever, cold and catarrh.

Ocimum Gratissimum

Ocimum gratissimum is an herbaceous plant belongs to the family Lamiaceae. The plant is indigenous to tropical areas especially in India and West Africa. It cultivated in Ceylon, South Sea Islands, Nepal, Bengal, Chittagong and Deccan. It is known by various names in different parts of the world. In India it has many vernacular names, the most commonly used ones are being Vriddhutulsi in Sanskrit, Ram tulsi in

Hindi, Nimmatulsi in Kannada, Lavangatulsi in Telugu and the common name is Clove Basil. Among the *Ocimum gratissimum* has extensively used in clinical practices throughout the world. Formulations of the leaf essential oil of *ocimum species gratissimum* (*Ocimum* oil) have been incorporated in a variety of bases as topical antiseptics and for use in the treatment of minor wounds, boils and pimples. The *ocimum species* are widely found in tropical and subtropical regions and commonly used as food spice and traditional herb. Studies on *ocimum species* were performed to investigate their therapeutic potentials to avoid the side effects by the administration of western medicines. The flowers and the leaves of this plant are rich in essential oils so it is used in the preparation of teas and infusions.

Ocimum gratissimum is used to through West Africa as anti - malarial and anti - convulsant. The crushed leaf juice is used in the treatment of convulsion, stomach pain and catarrh. Oil from the leaves have been founded to possess antiseptics, antibacterial and anti - fungal activities. The finding of Silva shows that the extracts of *osmium* are active in *in vitro* against human pathogenic dermatophytes¹³. The aim of present work therefore is to carry out phytochemical screening and elemental analysis of sample.



Ocimum Gratissimum

It is also used in the treatment of fungal infections, fever, cold and cough. The leaves are rubbed between the palms and sniffed as a treatment for blocked nostrils. They are also used for abdominal pains, sore eyes, ear infections, barrenness, fever, convulsions and tooth gargle, regulation of menstruation and as a cure for prolapsed of the rectum. In India, the whole plant has been used for the treatment of sunstroke, headache and influenza, as a diaphoretic, antipyretic and for its anti - inflammatory activities. The infusion of *O. gratissimum* leaves is used as pulmonary antiseptic, anti - tussive and antispasmodic. The medicinal value of the plant lies in its phytochemical composition, which produce definite physiological actions on the human body. The most important of this Phytochemical are alkaloids, tannins, steroids, triterpenoids, flavonoids, pentoses, hexoses, lipids, carbohydrates and phenolic compounds. The essential oils of *Ocimum* consists of compounds such as eugenol, methyl eugenol, cis - ocimene, trans - ocimene, pinene, camphor, germacrene - D, trans - caryophyllene, farnesene, 1 - bisabolene, *gratissimum*, thymol, p - cymene, γ terpene, trans sabinene hydrate, linalool, limonene, β - caryophyllene, α - terpineol, β - salinene, These essential oils are being used as

pharmaceutical agents because of their antimicrobial, antifungal, insecticidal, Leishmanicidal activity, ant malarial, Nematocidal activity, anti - diarrheals, analgesic, anti - mutagenic, antioxidant, antihypertensive, and antidiabetic and anticonvulsant activity. As the *Ocimum gratissimum* has proved medicinal properties it was chosen for the Synthesis and characterization of Silver nanoparticles. The synthesis and characterization of Silver nanoparticles from *Ocimum gratissimum* leaves extracts and their anti - helminthic activity, antibacterial and antifungal activity studies were presented in this chapter.

2. Materials and Method

The fresh leaves of plants *Ocimum gratissimum* and Coriander (*Coriandrum sativum* L.) were collected from narakoduru in Guntur district India. The selected plants were authenticated by Dr. Sandy Rani Asst. Professor, PG & Research Dept. of Botany Sims College of Pharmacy in Mangaldas Nagar Guntur. The leaves were dried at room temperature to avoid loss of chemical constituents and milled with the aid of grinding machine.

Selection of Experimental Worms

Indian adult earthworms (*Pheretima posthumous*) were used to carry out the experiment. *Pheretima posthumous* is commonly known as earthworm and were collected from water logged areas. *Ascaridia galli* is nematode were obtained from freshly slaughtered area. Both worms were identified by PG Department of Zoology, St Ann's junior college for girl's Barth Peta Gorantl. Worms were washed with normal saline to remove all fecal matter. The earthworms of 7 - 9 cm in length and 0.2 - 0.4 cm in width were used for all the experimental protocol. Ready availability, anatomical and physiological resemblance of *Pheretima posthumous* and *ascardia galli* made it to be used initially for *in vitro* evaluation of anthelmintic activity.

Preparation of Plant extract

The leaves of plants were thoroughly washed with tap water, dried at room temperature and transformed to coarse powder. The leaves powder was extracted with ethanolic separately by Soxhlet extraction method. Finally, the extract was evaporated and dried under vacuum to obtain thick sticky extract.

Drugs and Chemicals

Piperazine citrate [Actepar Syrup], Distilled water, Ethanol and were used during the experimental protocol. All the chemicals used are laboratory and analytical grade.

3. Experimental Work

Eight groups of approximately equal size worms consisting of one earthworm individually in each group were released into 10 ml of desired concentration of drug. The anti - helminthic assay was carried out as per the method with minor modification. The animals were divided into eight groups containing one earthworm in each different concentration of extracts and standard drug solution was poured in different Petri dishes. Observations were made for the time taken for paralysis (Paralysis was said to occur when worm did not revive in normal saline) and death (Time

for death of worms was recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (500ml), followed with their body colors fading away) [For evaluation of anthelmintic activity of *Coriandrum sativum* and *ocimum gratissimum* plants, group I was control, group II received standards drug (Piperazine citrate), group III, IV & V received extract of *ocimum gratissimum* while group VI, VII & VIII received extracts of *Coriandrum sativum* plant respectively. All the results were expressed in table 1.

Statistical Analysis¹⁴

The data presented as Mean \pm SEM. The activities of both the leaves extracts were compared with the control. All the extracts showed significantly higher duration of paralysis and death. Values of $P < 0.001$ were considered statistically significant.

4. Result and Discussion

Helminthiasis or infections with parasitic worms are pathogenic for human beings. Immature forms of the parasites invade human beings via the skin or gastrointestinal tract (GIT) and evolve into well differentiated adult worms that have characteristic tissue distribution. Anti-helminthic is drugs that may act locally to expel worms from the GIT or systemically to eradicate adult helminthes or development forms that invade organs and tissues.

The extracts of *Coriandrum sativum* and *ocimum gratissimum* plants were found to show anthelmintic

activity and fewer side effects when compared to standard drug. The extract of *ocimum gratissimum* concentration (50, 100, and 150 mg/ml) showed paralysis at 87.00, 42.33 and 32.33 min and death at 191.66, 146.00 and 88.33 min while extract of *Coriandrum sativum* concentration (50, 100, and 150 mg/ml) showed paralysis at 97.33, 72.00 and 48.66 min and death at 215.00, 152.00 and 109.66 min. The standard drug, Piperazine citrate showed paralysis at 21.66 min and death after 72.33 min at 15mg/ml concentration. From the above result, it is clear that extracts of *ocimum gratissimum* and *Coriandrum sativum* plants have significant anti-helminthic activity in dose dependent manner when compared with standard anti-helminthic drug. Data in the table 1 reveals that the extracts of *ocimum gratissimum* plant took the less time to cause paralysis of the earthworm than that of *coriandrum sativum* extract. It can be concluded that the active constituents responsible for anthelmintic activity present in the both extracts of *Coriandrum sativum* and *ocimum gratissimum* plants. Thus from results the *Coriandrum sativum* and *ocimum gratissimum* as an anthelmintic have been confirm as a plant extract displayed activity against the worm used in present study. The possible mechanism of the anti-helminthic activities of *Coriandrum sativum* and *ocimum gratissimum* cannot be explained on the basis of our present results. However, it may be due to its effect on inhibition of glucose uptake in the parasites and depletion of its glycogen synthesis while there need further study to isolate and revealed the active compound contained in the crude extracts of both plants as well as to establish mechanisms of action.

Table 1: Anti-helminthic Potency of *Coriandrum sativum* plant and *ocimum gratissimum*. (CSP&OG)

Treatment	group	Concentration (μ g/ml)	Time of paralysis (min)	Time of death (Min)
Control	I	-	-	-
Piperazine citrate (Std.)	II	15	21.66	72.33
Extract of <i>ocimum gratissimum</i>	III	50	87.00	191.66
	IV	100	42.33	146.00
	V	150	32.33	83.33
Extract of <i>coriandrum sativum</i>	VI	50	97.33	215.00
	VII	100	72.00	152.00
	VIII	150	48.66	109.66

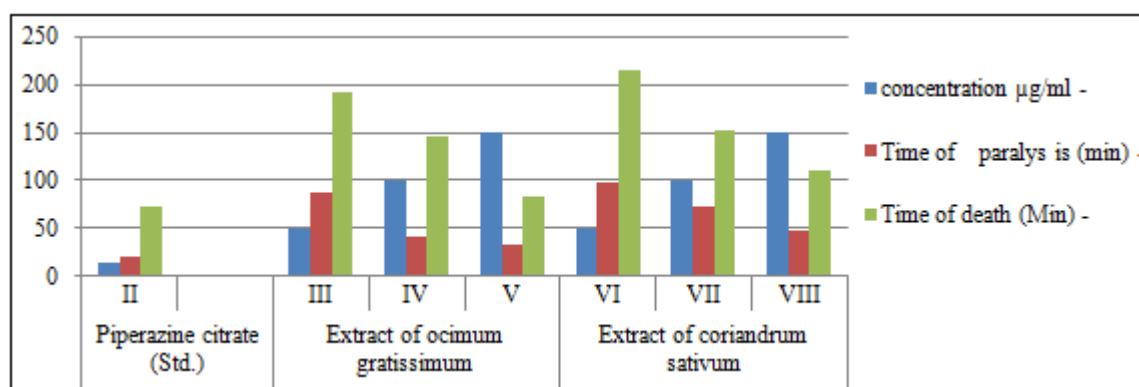


Figure 1: Anti-helminthic activity extract of *Coriandrum sativum* and *ocimum gratissimum* plant in Indian earthworm

Group - I control group, group - II standard piperazine citrate group, group - III IV & V extract of plant, CSP 50, 100, 150 mg/ml resp, VI, VII & VIII extract OGP having 50, 100, 150mg/ml resp

5. Conclusion

From the results, it can be concluded that both the extracts of *Coriandrum sativum* and *ocimum gratissimum* demonstrated dose dependent anti-helminthic activity when

compared with piperazine citrate and the revealed that the plant extract of ocimum gratissimum took less time to cause paralysis in earthworm than extract of coriandrum sativum, thus it can be concluded that extracts of ocimum gratissimum possess potent anti - helminthic activity. Thus from results the Coriandrum sativum and ocimum gratissimum as an anthelmintic have been confirm as a leaf extract displayed activity against the worm used in present study.

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